PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Philip Jordan THOMAS *et al.*

Serial No.: 09/775,051

Filed: January 31, 2001

For: PROTEIN/SOLUBILITY FOLDING ASSESSED BY STRUCTURAL COMPLEMENTATION

Group Art Unit: 1656

Examiner: D. Gunter

Atty. Dkt. No.: UTSD:703US/SLH

DECLARATION OF PHILIP JORDAN THOMAS

I, Philip Jordan Thomas, do declare that:

1. I am a U.S. citizen residing at 6958 Hillwood Lane, Dallas, Texas. I am an inventor on the above-captioned application.

2. Attached to this Declaration is a paper by Nixon & Benkovic entitled "Improvement in the efficiency of formyl transfer of a GAR transformylase hybrid enzyme," published in *Protein Engineering*, 13(5):323-327 (2000).

3. Nixon & Benkovic describe a fusion construct comprising sequences encoding the enzyme glycinamide ribonucleotide transformylase (GRT) and the α-peptide of β-galactosidase. This construct was transferred into cells expressing the ω-subunit of β-galactosidase (DH5-α).

When properly folded, the α and ω subunits combine to produce an active β-galactosidase enzyme which can process the substrate X-gal. The GRT sequences were mutated in an effort to find variants that had improved solubility. See abstract.

4. Claim 1 of the above-captioned application is reproduced below:

> 1. A method for assessing protein folding and/or solubility comprising:
>
> a) providing an expression construct comprising (i) a gene encoding fusion protein, said fusion protein comprising a protein of interest fused to a first segment of a marker protein, wherein said first segment has only systematic effects on the folding or solubility of the protein of interest, and (ii) a promoter active in said host cell and operably linked to said gene;
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> b) expressing said fusion protein in a host cell that also expresses a second segment of said marker protein, wherein said second segment is capable of structural complementation with said first segment; and
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> c) determining structural complementation,
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> wherein a greater degree of structural complementation, as compared to structural complementation observed with appropriate negative controls, indicates proper folding and/or solubility of said protein.

5. The method described by Nixon & Benkovic clearly is an assay designed to assess solubility. In addition, Nixon & Benkovic provide "an expression construct comprising (i) a gene encoding fusion protein, said fusion protein comprising a protein of interest fused to a first segment of a marker protein" (step a) and the step of "expressing said fusion protein in a host cell that also expresses a second segment of said marker protein, wherein said second segment is capable of structural complementation with said first segment." In addition, the also "determin[e] structural complementation." The example of β-galactosidase is provided in dependent claims 10-12.

6. Perhaps the only element not specifically recited is that "first segment has only systematic effects on the folding or solubility of the protein of interest" (step a). However, it is our experience that fusion of an enzyme to the α-subunit of β-galactosidase has little actual effect on the enzyme's folding.

7. Thus, to summarize, after a review of the attached Nixon & Benkovic paper, I believe that each element of claim 1 of the above-captioned application can be found in that paper.

8. I hereby declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date: _____ Dr. Philip J. Thomas: _____